Bioassay of *Clostera anastomosis* Granulosis virus

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Abstract: The second-instar healthy larvae of *Clostera. anastomosis* were collected in the artificial woodland of poplar in Shuangcheng Town, Heilongjiang Province, China. The dead larvae of *C. anastomosis* infected by granulosis virus (GV) of *Clostera anastomosis* were grinded to obtain GV. The GV viral pesticide was diluted to seven concentrations, 1.58×10^3PIB·mL^{-1}, 1.58×10^4 PIB·mL^{-1}, 1.58×10^5 PIB·mL^{-1}, 1.58×10^6 PIB·mL^{-1}, 1.58×10^7 PIB·mL^{-1}, 1.58×10^8 PIB·mL^{-1} and 1.58×10^9 PIB·mL^{-1} and the fresh poplar leaves were dipped in the seven concentrations liquids to feed the larvae. After nine days the mortality of larvae was investigated. The minimum corrected mortality (7.32%) of larvae was observed at concentrations of 1.58×10^3PIB·mL^{-1} and the maximal mortality (97.36%) was observed at concentration of 1.58×10^9 PIB·mL^{-1}. The regression equation between the logarithm of the virus concentration and the mortality was y=1.946+0.558x. The LC_{50} was 2.97×10^5PIB·mL^{-1}. The LT_{50} for the virus concentration of 1.58×10^5 , 1.58×10^6, 1.58×10^7, 1.58×10^8, 1.58×10^9 PIB·mL^{-1} were 8.55d, 6.89d, 5.9d, 4.65d, and 4.08d, respectively, shorting gradually with the concentration increasing. It is concluded that the toxicity of *Clostera anastomosis* GV is very strong and as a kind of insecticides it has big potential in practical application.

Key words: *Clostera anastomosis*; Larvae; *G*ranulosis virus, Virulence; Bioassay
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Introduction

Clostera anastomosis is a worldwide defoliating insect, belonging to Notodontidate of Lepidoptera, and distributes in Europe, North America and China (from Great Xing'an Mountains in North to Guangdong Province in South) (Guo 1998). It develops different generations per year in different regions, damage poplar, willow and birch, and its destructive result is rather serious. In 1986, *C. anastomosis* was very epidemic in some areas of the Yingchun town, Northeast China, and the outbreak area reached 467 hm^2.The whole forest infested by the insect seemed to be burnt. In the second year, the growth of the infested trees declined and became rotten easily. The seriously infested trees can be caused to death (Li 1989; Yang 2003). In 1990-1994, *C. anastomosis* pest broke out in the poplar artificial woodland in Heilongjing, Jilin, Liaoning provinces and Inner Mongolia (Wang 1998), and also caused serious damage to the forests.

Presently two kinds of virus have been isolated from the dead larvae of *C. anastomosis*, nuclear polyhedrosis virus and granulosis virus, vairing with regions. In the forestry centre of Xinhua and Anguang of Jilin Province, Liu Bo (1998) obtained The nuclear polyhedrosis virus from dead larvae of *C. anastomosis* by separating and purifying and tested its virulence. Granulosis virus is one of the most potential insect-virus and its application is quite extensive. It can accumulate in the environment, which is important to control the occurrence of pest cluster (Kolodny 1993). The infection of granulosis virus on the larvae of Lepidoptera is very strong.

In our study, granulosis virus was obtained from the diseased larvae of *C. anastomosis* and its virulence was tested, with a purpose to provide important basis for the development of insecticide to control of *C. anastomosis*

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Materials and methods

Larva

The second-instars healthy larvae of *C. anastomosis* were collected in the artificial woodland of poplar in Shuangcheng Town, Heilongjiang Province and reared in the laboratory on fresh poplar leaves. The larvae were hungered for 24 h before experiment and then were put into the insect cages (Chen 1994). Totally seven testing group and one control were arranged.

Collection of insects and purification of Granulosis virus

The dead larvae of *C. anastomosis* infected by granulosis virus (GV) of *C. anastomosis* were collected and grinded. The suspension was filtrated and centrifuged thrice at 800 *g* for 30 min. The supernatant was centrifuged thrice at 12000 *g* for 30 min at 4 °C. Then the deposition was the purified GV (Zhang 2001; Liu 1994; Martins 2005). The GV viral pesticide was taken count by arithmometer and was diluted to seven concentrations, 1.58×10^3PIB·mL^{-1}, 1.58×10^4 PIB·mL^{-1}, 1.58×10^5 PIB·mL^{-1}, 1.58×10^6 PIB·mL^{-1}, 1.58×10^7 PIB·mL^{-1}, 1.58×10^8 PIB·mL^{-1} and 1.58×10^9 PIB·mL^{-1} (Jing 1995; Lu 1997; Wang 1994; Zu 1997).

Infecting method

We first put the hungered larvae into the insect cages, and then dip the fresh leaves into the seven concentrations liquids separately. Three repeats were made for one concentration. The leaves were cooled in doors and put inside the cages. The control group was dealt with water in the same conditions. Meanwhile the temperature and the humidity of the laboratory were consistent during the experiment (Lin 1995).

Checking method

On August third, the larvae were infected with virus. After 24 hours the insects ate all leaves and the nontoxic leaves were put

into the cages (Zhou 1999). They were fed with poplar leaves that were changed at least every 2 days (Agata 2005). The number and cause of death for *C. anastomosis* were investigated and recorded everyday. The experiment was conducted for nine days.

Statistical analysis

The mortality of the larva of *C. anastomosis* was calculated. Corrected mortality = (dealt mortality－compared mortality)/ (1 －compared mortality) ×100%. The corrected mortality was converted to probability according to the statistics. The regression equation about the logarithm of concentration and the probability of death was obtained by the ware of SPSS (SPSS Inc.). Meanwhile, LC_{50} and the limit of 95% were analyzed.

Results and discussion

Effect of different concentrations on the 2rd-instar larvae of *C. anastomosis*

The death results of *C. anastomosis* larvae infected with different concentrations of viral pesticide are shown in Table. The maximal mortality was 97.36% and the minimum was 7.32% after nine days.

Table 1. Mortality of the 2rd-instars larvae of *Clostera anastomosis* infected with *Clostera anastomosis* GV

GV Concentrations (PIB·mL^{-1})	Number of Insect (Head)	Number of death (Head)	Corrected Mortality (%)	Probability
1.58×10^{3}	77	40	7.32	3.55
1.58×10^{4}	65	39	22.85	4.25
1.58×10^{5}	79	60	53.61	5.09
1.58×10^{6}	77	64	67.44	5.45
1.58×10^{7}	67	62	85.61	6.06
1.58×10^{8}	85	82	93.19	6.48
1.58×10^{9}	73	72	97.36	6.94

The analytical table was obtained by the ware of SPSS. The regression equation was y=1.946+0.558x. From Table 2, it is clear that *F* was equal to 346.874 and the probability was less than 0.001. This result indicated that the effect of different concentrations on mortality was notable. The linear relation between *x* and *y* was logical. From Table 3, the related coefficient was 0.993. T was equal to 18.625 and its probability was less than 0.001. This indicated that the difference between regression coefficient and zero was notable. Calculated from the regression equation, LC_{50} was 2.97×10^{5} PIB·mL^{-1}. The upper limit of 95% was 5.75×10^{5} PIB·mL^{-1} and the under was 1.54×10^{5} PIB·mL^{-1}.

Table 2. Notability-test of regression equation (Analysis of Variance)

Source	DF	SS	MS	F	Sig.
Between	1	8.724	8.724	346.874	.000
Groups	5	0.126	0.025		
Total	6	8.849			

The line relation between *x* and *y* was shown in Figure 1. It is clear that the mortality of larvae increases with the increase of concentrations. The higher the concentration is, the higher the mortality is.

Table 3. Notability-test of regression coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	*t*	*Sig.*
	B	Std. Error	Beta		
(Constant)	1.946	0.195		9.969	0.000
X	0.558	0.030	0.993	18.625	0.000

Notes: The constant was 1.946 and the regression coefficients was 0.558

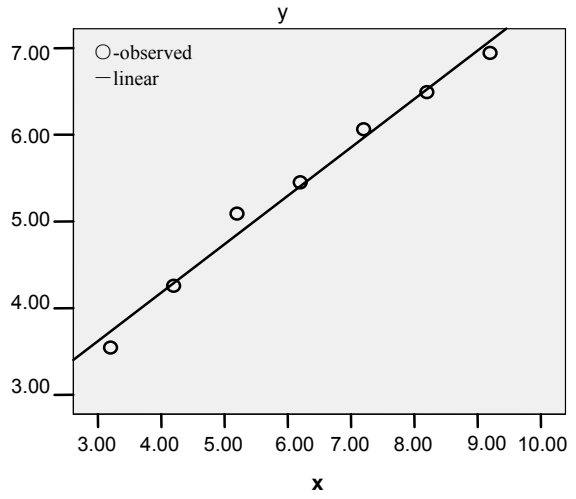

Fig. 1 The linear relation between the logarithm of concentration and the probability of death

Notes: The x-axis stands for the logarithm of concentration and y stands for the probability of death

Effect of time on the 2rd-instar larva of *C. anastomosis*

The 2rd-instars larvae of *C. anastomosis* were infected with five concentrations (1.58×10^{5} PIB/ml, 1.58×10^{6} PIB/ml, 1.58×10^{7} PIB/ml, 1.58×10^{8} PIB/ml, 1.58×10^{9} PIB/ml) of GV viral pesticide. Results showed that the mortality of larvae was over 50% and The LT_{50} was shorting gradually along with the concentration increasing. The LT_{50} of the higher concentration was 2 to 4 days earlier than the lower concentration (Table 4).

Table 4. The statistics between the days and probability of death

GV Concentration (PIB/ml)	Regression equation	Related coefficient	LT_{50} (d)	The limit of 95%(d)	
				Upper Limit	Under Limit
1.58×10^{9}	y=0.189+1.178x	0.983	4.08	4.55	3.62
1.58×10^{8}	y=2.418+0.530x	0.932	4.65	6.36	3.38
1.58×10^{7}	y=2.298+0.456x	0.959	5.90	6.62	5.16
1.58×10^{6}	y=2.368+0.382x	0.847	6.89	8.43	5.53
1.58×10^{5}	y=2.471+0.286x	0.948	8.55	10.45	7.23

In the experiment of testing virulence, different species of insect, ages of larvae may influence the result directly as well as the virus. When the conditions were the same, the virulence descended along with the age increasing. This study showed that the mortality of the second-instars larva of *Clostera anastomosis* was just related with the concentration as well as time. In the certain scope, the mortality rose with the increasing of the concentration and the lasting of the time. The maximal corrected mortality reached to 97.36%. It indicated that the toxicity of this virus is very strong and the young larvae are easily infected. The results of this study may provide an important basis for the

development of insecticide of *Clostera anastomosis* and application in the field.

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